


## RESEARCH ARTICLE

# Environmentally independent selection for hybrids between divergent freshwater stickleback lineages in semi-natural ponds

Cameron Marshall Hudson<sup>1,2,3</sup>  | Maria Cuenca Cambronero<sup>1,4</sup> | Marvin Moosmann<sup>1,2</sup> | Anita Narwani<sup>3</sup> | Piet Spaak<sup>3</sup> | Ole Seehausen<sup>1,2</sup> | Blake Matthews<sup>1</sup><sup>1</sup>Department of Fish Ecology and Evolution, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Center of Ecology, Evolution and Biochemistry, Lucerne, Switzerland<sup>2</sup>Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland<sup>3</sup>Department of Aquatic Ecology, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Zürich, Switzerland<sup>4</sup>Aquatic Ecology Group, University of Vic, Central University of Catalonia, Vic, Spain**Correspondence**Cameron Marshall Hudson, Department of Fish Ecology and Evolution, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Center of Ecology, Evolution and Biochemistry, Seestrasse 79, CH-6047 Kastanienbaum, Lucerne, Switzerland.  
Email: [cameron.hudson@eawag.ch](mailto:cameron.hudson@eawag.ch)**Funding information**

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**Abstract**

Hybridization following secondary contact of genetically divergent populations can influence the range expansion of invasive species, though specific outcomes depend on the environmental dependence of hybrid fitness. Here, using two genetically and ecologically divergent threespine stickleback lineages that differ in their history of freshwater colonization, we estimate fitness variation of parental lineages and hybrids in semi-natural freshwater ponds with contrasting histories of nutrient loading. In our experiment, we found that fish from the older freshwater lineage (Lake Geneva) and hybrids outperformed fish from the younger freshwater lineage (Lake Constance) in terms of both growth and survival, regardless of the environmental context of our ponds. Across all ponds, hybrids exhibited the highest survival. Although wild-caught adult populations differed in their functional and defence morphology, it is unclear which of these traits underlie the fitness differences observed among juveniles in our experiment. Overall, our work suggests that when hybrid fitness is insensitive to environmental conditions, as observed here, introgression may promote population expansion into unoccupied habitats and accelerate invasion success.

**KEYWORDS**

adaptation, hybridization, invasive species, threespine stickleback

## 1 | INTRODUCTION

Range-expanding species often develop into ecologically and genetically divergent lineages as they spread to, and colonize new locations. Human activities can influence the spread of such species and, in some cases, facilitate secondary contact between divergent lineages that might otherwise have remained separate. In circumstances where genetically variable populations come into secondary contact,

we are able to investigate the fitness consequences of hybridization and their influence on the process of adaptation during colonization. There are several possible ecological and evolutionary outcomes that might follow secondary contact. If the lineages become strongly reproductively isolated species prior to secondary contact (e.g., ring species: Irwin et al., 2001), then these species might either coexist together or competitively exclude one another, depending on their order of arrival at a local site and the ecological differences between

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them. However, secondary contact more commonly occurs prior to the evolution of strong reproductive isolation, meaning that the specific outcomes will depend on the extent of introgressive hybridization between lineages, the environmental dependence of hybrid fitness, and any subsequent evolution of reproductive isolation (i.e., reinforcement of reproductive isolation or hybrid speciation) (Epifanio & Philipp, 2000). In relation to invasive species, it is possible that human activities influence the spatial spread, the environmental conditions across their range, and the timing and location of secondary contact. It is well known that the degree of invasiveness can evolve rapidly during range expansion (Hill et al., 2011; Hudson et al., 2020; Perkins et al., 2013; Phillips et al., 2008, 2010; Wagner et al., 2017), but less is known about how secondary contact during invasions affects performance and hence range expansion.

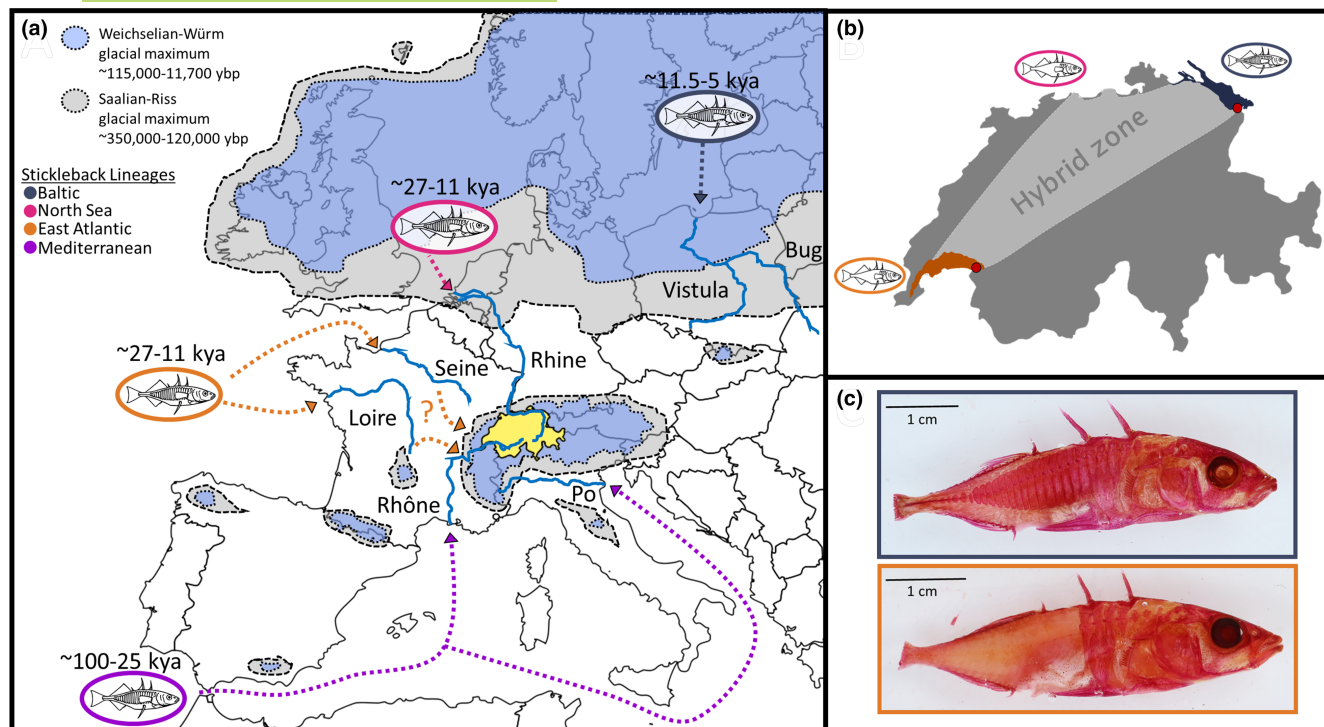
The extent and environmental context of hybridization can potentially influence the outcomes of secondary contact between divergent lineages in natural populations, including the spread of invasive species. The impact of hybridization on the fitness of invasive species has been extensively studied in plants (Ellstrand & Schierenbeck, 2006; Hahn & Rieseberg, 2017; Moody & Les, 2002; Prentis et al., 2008), and to a lesser degree in animals (Drake, 2006; Kolbe et al., 2004; Smyser et al., 2020; Strait et al., 2021; Wagner et al., 2017). One relevant hypothesis is the 'catapult effect', which suggests that rapid growth of  $F_1$  populations, for example, due to heterosis, may promote the establishment of invasive species (Drake, 2006). Heterosis is a well-known phenomenon by which first-generation hybrids exhibit enhanced growth or fitness relative to both parental lineages (also referred to as hybrid vigour), and is commonly observed in agricultural settings (Labroo et al., 2021), but can also be seen in natural populations (see Birchler et al., 2010; Chen, 2013). Existing theory often attributes heterosis to evolutionary genetic mechanisms, such as the masking of deleterious recessive alleles (i.e., via dominance) or the emergence of novel combinations of alleles (i.e., overdominance) (Fiévet et al., 2018; Hochholdinger & Hoecker, 2007; Lippman & Zamir, 2007; MacPherson et al., 2022; Whitlock et al., 2000). Such mechanisms could help explain why hybridization between inbred lineages leads to enhanced fitness of hybrids, but similar predictions for outbred natural populations are less clear. Importantly, previous work has shown that heterosis can be environment dependent (Thompson et al., 2022; Wagner et al., 2021), for example, the heterosis of hybrid corn strains can depend on the microbial community in the soil. In other cases, hybridization among populations can result in heterosis (Thompson & Schluter, 2022), but hybridization between closely related species might be counteracted in certain environmental contexts if hybrid individuals with mismatching traits perform poorly (Arnegard et al., 2014; Selz & Seehausen, 2019). Such environment-dependent heterosis, either within or between lineages, could influence the invasion dynamics of outbred populations following secondary contact.

Threespine stickleback (*Gasterosteus aculeatus* species complex) is a useful model for studying how the outcomes of secondary contact might influence subsequent invasion dynamics in natural populations. There is ample work on the adaptation of stickleback to freshwater environments, adaptive population divergence of these populations

as they expand their range and colonize divergent types of water bodies, and on the role of secondary contact for ecological speciation (Coyne & Orr, 2004; Hendry et al., 2009; Lackey & Boughman, 2017; Nosil, 2012; Ravinet et al., 2013; Schluter, 2000). Many studies have investigated sexual and ecological selection against hybrids (Arnegard et al., 2014; Gow et al., 2007; Hanson et al., 2016; Hatfield & Schluter, 1999; Keagy et al., 2016; Kitano et al., 2007; Raeymaekers et al., 2010; Thompson et al., 2021; Vamori et al., 2000) as intrinsic reproductive isolation barriers are typically weak or non-existent in stickleback (Lackey & Boughman, 2017), and hybridization is common. Much of this work has been in the context of hybridization between sympatric or parapatric species pairs (see Hendry et al., 2009 and citations within), but rarely between anciently divergent allopatric freshwater lineages (though see Dean et al., 2019; Ravinet et al., 2013). Investigating secondary contact between lineages that differ in colonization history could thus provide insight into the role of hybridization in population divergence and range expansion.

European freshwater stickleback populations are composed of multiple anciently divergent lineages that differ markedly in colonization age and history (Fang et al., 2018, 2020; Mäkinen et al., 2006; Mäkinen & Merilä, 2008). In some cases, there have been secondary contact between such lineages, but the implications for further range expansion are unclear. The perialpine region of Switzerland north of the Alps is a zone of recent secondary contact between several divergent lineages. For example, two of these lineages are represented by populations in Lake Constance and Lake Geneva respectively, that were both introduced in the late 1800s (see Hudson, Lucek, et al., 2021 for a review). Currently, stickleback has a broad distribution in Switzerland, including the Rhine, Rhône, Po, and Aare drainages, and an extensive zone of secondary contact between three lineages has formed throughout the central Swiss plateau (Lucek, 2016; Lucek et al., 2010; Roy et al., 2015). Here we are interested in two of these Swiss stickleback lineages, the western European *G. gymnotus* that was introduced to Lake Geneva, and the northern European *G. aculeatus* that was introduced to Lake Constance (hereafter referred to as "Constance" and "Geneva" lineages). Though both lineages have been present in Switzerland for ~150 years, they are vastly different with respect to their age of freshwater colonization (Figure 1). The western European lineage colonized modern-day France from the East Atlantic during the late Pleistocene ~27–11 Kya (undergoing possible secondary contact with the even much older Mediterranean lineage in the upper Rhône, see Fang et al., 2018; Mäkinen et al., 2006; Mäkinen & Merilä, 2008), while the northern European lineage colonized the Baltic sea drainage basin following the deglaciation of central Europe ~17–5 Kya (Fang et al., 2018, 2020). Thus, the Geneva lineage has a long evolutionary history of adaptation to life in freshwater (e.g., see Figure 1).

Since these divergent lineages differ in both their colonization and evolutionary histories (Hudson, Lucek, et al., 2021), we wanted to explore the relative fitness of the lineages and their hybrids in contrasting environmental and food web conditions. Based on the observed differences in natural history between the two populations (i.e., Lake Constance fish are abundant in open water environments while Lake Geneva fish are largely confined to littoral habitats) we predicted that



**FIGURE 1** Colonization history of several prominent stickleback lineages in Central Europe with an overlay of the extent of glaciers during the last two maxima. Panel (a) depicts the estimated colonization routes and times for four stickleback lineages; estimated times since colonization were interpreted by the authors from previous studies by (Mäkinen et al., 2006; Mäkinen & Merilä, 2008; Fang et al., 2018, 2020). The orange lines with a question mark indicate a possible, but as yet unconfirmed zone of secondary contact, with signs of mitonuclear discordance occurring in some Rhône populations. Panel (b) highlights the zone of hybridization in Switzerland and the lakes from which our samples were collected (Lake Geneva in orange, Lake Constance in blue). Panel (c) shows two alizarin red stained adult male stickleback; a fully plated male from Lake Constance (Baltic lineage) and a low plated male from Lake Geneva (East Atlantic lineage).

each lineage would perform better in an environment where more resembled their source habitat; Constance in an oligotrophic habitat, Geneva in a eutrophic habitat. Therefore, in this study, we generated contrasting environments in the form of artificially eutrophic and oligotrophic semi-natural ponds. We then introduced lab-bred populations of each lineage, and hybrids between them, to quantify juvenile growth and survival in these environments. As these two lineages occupy different niches in the wild (Hudson, Lucek, et al., 2021) and exhibit differences in survival in mesocosm experiments (Best et al., 2017) we first collected wild adult fish to compare their morphology of ecologically relevant traits, and to breed juveniles for the pond experiment. Using the lab-reared offspring juveniles, we assessed how these lineages and their hybrids perform under contrasting environmental conditions, simulating a situation of recent secondary contact.

## 2 | MATERIALS AND METHODS

### 2.1 | Study system – two stickleback lineages with contrasting evolutionary histories

The Geneva and Constance lineages differ markedly in their time since freshwater colonization (Figure 1) and in many ecologically relevant traits. When compared to the Constance lineage, Lake Geneva fish

have fewer bony lateral plates (i.e., only the structural plates), deeper bodies, shorter gill rakers, and smaller eyes (Berner et al., 2010; Lucek et al., 2010, 2013), which collectively suggest more benthic or littoral feeding behaviour and habitat utilization (Kottelat & Freyhof, 2007). Furthermore, increased *FADS2* copy numbers in the Geneva lineage (Ishikawa et al., 2019) and fatty acid synthesis ability (Hudson, Ladd, et al., 2021) allow them to produce more omega-3 fatty acids from a diet of benthic invertebrates (which are lower in omega-3 fatty acids in comparison to zooplankton; Twining et al., 2021), and by inference, perhaps they survive better in nutrient-poor environments such as streams (Ishikawa et al., 2021). Under experimental conditions, fish from the Geneva lineage exert a stronger effect on the benthic invertebrate community in mesocosms (Best et al., 2017), and consume more benthic crustaceans such as isopods than Constance lineage fish (Moosmann et al., 2023). In contrast, fish from the Constance lineage are efficient planktivores (Best et al., 2017; Schmid et al., 2019), and wild-caught fish from Lake Constance has a predominantly planktonic diet (Bretzel et al., 2021; Hudson, Lucek, et al., 2021). The population in Lake Constance has garnered particular interest from ecologists and conservation biologists because of their rapid increase in abundance in the pelagic zone of the lake over the past decade (Alexander et al., 2016; Eckmann & Engesser, 2019). By comparison, the population in Lake Geneva is at a much lower abundance, and is not very common in the open water habitats (Alexander & Seehausen, 2021).

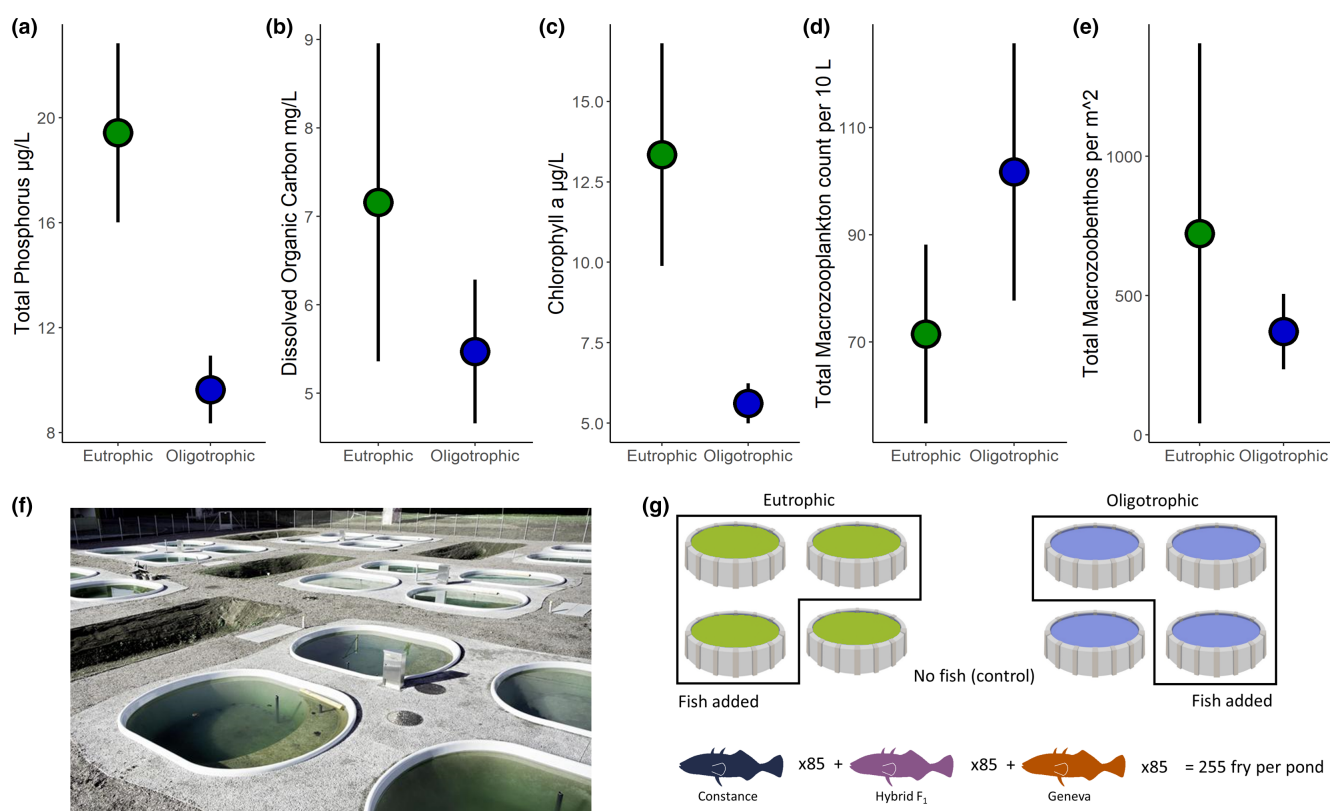
## 2.2 | Fish collection, breeding, and husbandry

From May to June of 2019, during the stickleback breeding season, we collected adults in breeding condition (visibly gravid females, and males with red nuptial coloration) from Lake Geneva (Le Grand Canal; 46.396770° N, 6.887284° E) and Lake Constance (Jägershaus Marina; 47.490264° N, 9.547921° E) using steel minnow traps. These fish were brought back to our lab and euthanized on the same day of capture with 1g/L ethyl 3-aminobenzoate methanesulfonate (MS-222) prior to artificial spawning. To produce clutches in the lab, mature eggs were stripped from female stickleback into Petri dishes containing water, and mixed with milt from macerated testes dissected out of the body cavities of males. Each clutch had one sire and one dam. Using this method we produced 79 clutches of successfully fertilized eggs in the following pairings: Geneva ♂ × Geneva ♀ (G × G) 24, Constance ♂ × Geneva ♀ (C × G) 14, Geneva ♂ × Constance ♀ (G × C) 13, Constance ♂ × Constance ♀ (C × C) 28. Additional clutches were produced, but these either failed to fertilize or died prior to hatching and thus they were not included in the experiment. From the surviving clutches, fertilized eggs were separated using a fine-tip dissection probe, and egg clutches were then maintained in individual mesh containers within a flow-through incubator at 15°C. Incubators were checked daily during

this time, and unfertilized, dead, or fungal-infected eggs were removed from the clutch using a pipette to ensure health of the remaining eggs. Eggs typically hatched 10 days post-fertilization at this temperature. After juvenile stickleback was free-swimming and had resorbed their yolk sac (3–4 days post-hatching), clutches were mixed together by parental origin (i.e., G × G, hybrid, and C × C) and transferred into 100L aquaria. This was done to generate a large, phenotypically diverse population of each lineage that could be reared in a common garden. These juvenile fish were reared on a diet of live artemia nauplii until they were large enough to consume finely chopped frozen chironomid larvae. We performed all fish collection and husbandry in accordance with permits obtained from the Swiss cantons of St. Gallen and Vaud. We obtained animal care permits and approval for this experiment from the Swiss federal veterinary office and the cantons of Lucerne and Zurich.

## 2.3 | Experimental ponds

Eawag's research facility in Dübendorf, Switzerland (47.4038° N, 8.6098° E) houses 36 identical 15 m<sup>3</sup> outdoor experimental ponds (Figure 2). These ponds are 4 m in diameter, 1.5 m deep, and have a shallow 0.5 m section (Figure S1). They were 3 years post-filling



**FIGURE 2** Panels (a–e): environmental conditions and prey densities of our experimental ponds prior to fish introduction. Mean values with standard error for the eutrophic (green) and oligotrophic (blue) ponds are shown. Panel (f) depicts our experimental ponds prior to manipulation. From these ponds we randomly selected eight (four per environmental treatment), to which we introduced fish to six (three per treatment). Panel (g) outlines our experimental design, where each of the selected ponds received 255 fish fry (85 of each lineage) at the onset of the experiment. Five hundred and ten fish of each lineage were collected from each of the mixed family tanks (within lineage) and then split into six groups to ensure that families were randomized and equally represented in each pond.



at the time of our experiment. Prior to our study, these ponds were part of a long-term ecosystem manipulation experiment (see Lürig et al., 2021; Narwani et al., 2019 for details) and were inoculated with zooplankton and phytoplankton from Lake Greifensee (47.3478° N, 8.6793° E) as well as macroinvertebrates from Lake Lucerne (47.0136° N, 8.4372° E). In addition, certain ponds had a cumulative history of nutrient loading over the 3 years prior to the experiment, where phosphorus and nitrogen were added in the form of KNO<sub>3</sub> and K<sub>2</sub>HPO (Lürig et al., 2021). Data on water chemistry and the plankton community were collected weekly to document

ecosystem responses to nutrient loading over the course of the ecosystem manipulation experiment. Samples of the benthic invertebrate community were collected once per year, in the autumn. The eight ponds used in the present experiment were divided into two treatment categories, those with a history of eutrophication (eutrophic), where nutrients were added, and those without (oligotrophic) (Figure 2). After inoculation, the ponds are open to the environment and were subsequently colonized by aquatic invertebrates with airborne adult stages. In this study, we used data collected by (Narwani et al., 2019) and (Lürig et al., 2021) on pond environmental

Mouthparts	Sum Sq	DF	F value	p-Value
Dorsal mouth protrusion				
Head length	1.31E+00	1, 160	48.56	<b>&lt;0.001</b>
Lineage	1.46E-02	1, 160	0.54	0.46
Sex	8.00E-03	1, 160	0.30	0.59
Head length × Lineage	1.47E-02	1, 160	0.55	0.46
Head length × Sex	1.28E-02	1, 160	0.47	0.49
Lineage × Sex	1.39E-01	1, 160	5.16	<b>0.024</b>
Head length × Lineage × Sex	1.35E-01	1, 160	4.99	<b>0.027</b>
Lower jaw length				
Head length	8.01E-01	1, 160	155.51	<b>&lt;0.001</b>
Lineage	1.60E-02	1, 160	3.10	0.08*
Sex	6.54E-03	1, 160	1.27	0.26
Head length × Lineage	1.35E-02	1, 160	2.62	0.11
Head length × Sex	4.88E-03	1, 160	0.95	0.33
Lineage × Sex	1.25E-03	1, 160	0.24	0.62
Head length × Lineage × Sex	1.04E-03	1, 160	0.20	0.65
Gape width				
Head length	9.99E-01	1, 160	100.23	<b>&lt;0.001</b>
Lineage	1.76E-03	1, 160	0.18	0.67
Sex	7.55E-03	1, 160	0.76	0.39
Head length × Lineage	1.54E-03	1, 160	0.15	0.69
Head length × Sex	9.20E-03	1, 160	0.92	0.33
Lineage × Sex	3.74E-02	1, 160	3.75	0.054*
Head length × Lineage × Sex	3.74E-02	1, 160	3.75	0.054*
Suction index				
Lineage	2.08E+00	1, 164	14.81	<b>&lt;0.001</b>
Sex	1.35E+00	1, 164	9.64	<b>&lt;0.001</b>
Lineage × Sex	6.60E-01	1, 164	4.71	<b>&lt;0.001</b>
Displacement advantage				
Lineage	1.32E-01	1, 164	6.20	<b>0.001</b>
Sex	1.27E-01	1, 164	5.96	<b>0.002</b>
Lineage × Sex	5.00E-03	1, 164	0.22	0.62
Opercular 4-bar KT				
Lineage	2.00E-02	1, 157	0.62	0.43
Sex	7.00E-02	1, 157	1.77	0.19
Lineage × Sex	1.60E-01	1, 157	4.10	<b>0.044</b>

**TABLE 1** Statistical tests for a comparison of mouthpart traits between wild-caught adult fish from Lake Constance and Lake Geneva.

Note: The table shows sums of squares (Sum Sq), degrees of freedom (DF), F-values and p-values (bold =  $p < 0.05$ , \* =  $p < 0.1$ ) from Type III ANOVAs with standard length, lineage, and sex as factors. In the case of functional traits, standard length was not included in the model as the functional traits are composed of other length measures and effectively size corrected.

conditions, invertebrate, and zooplankton abundances in our analysis, along with that collected during the experiment. On July 16th 2019, when juvenile stickleback were 32–43 days old (post-hatching),

we randomly selected six of the eight ponds (including three of each treatment) and introduced 255 juveniles into each (85 per cross type – G×G, hybrid, and C×C), keeping the two remaining ponds

**TABLE 2** Statistical tests for a comparison of non-mouthpart morphological traits between wild-caught adult fish from Lake Constance and Lake Geneva.

Morphological traits	Sum Sq	DF	F value	p-Value
Standard length				
Lineage	3.40E-01	1, 176	92.97	<b>&lt;0.001</b>
Sex	6.00E-02	1, 176	16.86	<b>&lt;0.001</b>
Lineage×Sex	2.00E-02	1, 176	6.03	<b>0.015</b>
Body width				
Standard length	6.91E-01	1, 172	165.99	<b>&lt;0.001</b>
Lineage	3.82E-03	1, 172	0.917	0.34
Sex	1.12E-02	1, 172	2.69	0.10
Standard length×Lineage	5.95E-03	1, 172	1.43	0.23
Standard length×Sex	1.32E-02	1, 172	3.18	0.076*
Lineage×Sex	8.33E-03	1, 172	2.00	0.16
Standard length×Lineage×Sex	8.53E-03	1, 172	2.05	0.15
Lateral head length				
Standard length	4.28E-01	1, 172	432.93	<b>&lt;0.001</b>
Lineage	7.10E-04	1, 172	0.71	0.40
Sex	6.00E-05	1, 172	0.06	0.80
Standard length×Lineage	5.60E-04	1, 172	0.57	0.45
Standard length×Sex	2.70E-04	1, 172	0.28	0.60
Lineage×Sex	0.00E+00	1, 172	0.00	0.96
Standard length×Lineage×Sex	1.00E-05	1, 172	0.01	0.92
Epaxial height				
Standard length	3.68E-02	1, 172	1.26	0.26
Lineage	1.54E-01	1, 172	5.25	<b>0.02</b>
Sex	6.10E-03	1, 172	0.21	0.65
Standard length×Lineage	1.58E-01	1, 172	5.39	<b>0.02</b>
Standard length×Sex	6.40E-03	1, 172	0.22	0.64
Lineage×Sex	6.05E-02	1, 172	2.07	0.15
Standard length×Lineage×Sex	5.63E-02	1, 172	1.92	0.17
Eye width				
Standard length	1.82E-01	1, 172	43.96	<b>&lt;0.001</b>
Lineage	1.47E-03	1, 172	0.36	0.55
Sex	8.70E-04	1, 172	0.21	0.65
Standard length×Lineage	1.21E-03	1, 172	0.29	0.59
Standard length×Sex	1.42E-03	1, 172	0.34	0.56
Lineage×Sex	4.52E-03	1, 172	1.09	0.30
Standard length×Lineage×Sex	4.38E-03	1, 172	1.06	0.30
Body depth				
Standard length	2.76E-01	1, 172	143.68	<b>&lt;0.001</b>
Lineage	7.00E-05	1, 172	0.04	0.85
Sex	5.10E-03	1, 172	2.65	0.11
Standard length×Lineage	1.40E-04	1, 172	0.07	0.79
Standard length×Sex	4.36E-03	1, 172	2.27	0.13
Lineage×Sex	1.00E-04	1, 172	0.05	0.82
Standard length×Lineage×Sex	3.00E-05	1, 172	0.02	0.90

Note: The table shows sums of squares (Sum Sq), degrees of freedom (DF), F-values and p-values (bold= $p < 0.05$ ) from Type III ANOVAs with standard length, lineage, and sex as factors.

\* indicate a marginally significant effect (i.e.  $p < 0.1$ ).

Defence traits	Sum Sq	DF	F value	p-Value
Plate number				
Lineage	2.16E+04	1, 176	1478.94	<b>&lt;0.001</b>
Sex	1.20E+01	1, 176	0.841	0.364
Lineage×Sex	0.00E+00	1, 176	0.018	0.891
First dorsal spine length				
Standard length	1.03E-02	1, 172	34.04	<b>&lt;0.001</b>
Lineage	0.00E+00	1, 172	0.89	0.35
Sex	8.24E-03	1, 172	0.00	1.00
Standard length×Lineage	1.00E-05	1, 172	0.71	0.40
Standard length×Sex	9.58E-03	1, 172	0.00	0.97
Lineage×Sex	9.27E-03	1, 172	0.82	0.37
Standard length×Lineage×Sex	2.00E+00	1, 172	0.80	0.37
Second dorsal spine length				
Standard length	3.70E-01	1, 172	36.60	<b>&lt;0.001</b>
Lineage	2.04E-02	1, 172	2.02	0.16
Sex	1.42E-02	1, 172	1.41	0.24
Standard length×Lineage	1.64E-02	1, 172	1.62	0.20
Standard length×Sex	1.49E-02	1, 172	1.47	0.23
Lineage×Sex	6.00E-04	1, 172	0.06	0.81
Standard Length×Lineage×Sex	5.30E-04	1, 172	0.05	0.82
Pelvic spine length				
Standard length	3.08E-01	1, 172	25.97	<b>&lt;0.001</b>
Lineage	2.26E-02	1, 172	1.90	0.17
Sex	2.24E-03	1, 172	0.19	0.66
Standard length×Lineage	1.66E-02	1, 172	1.40	0.24
Standard length×Sex	2.64E-03	1, 172	0.22	0.64
Lineage×Sex	6.25E-02	1, 172	5.27	<b>0.02</b>
Standard length×Lineage×Sex	6.43E-02	1, 172	5.42	<b>0.02</b>
Ascending process width				
Standard length	5.24E-01	1, 172	38.64	<b>&lt;0.001</b>
Lineage	7.42E-03	1, 172	0.55	0.46
Sex	1.67E-02	1, 172	1.23	0.27
Standard length×Lineage	7.83E-03	1, 172	0.58	0.45
Standard length×Sex	1.63E-02	1, 172	1.20	0.27
Lineage×Sex	7.40E-04	1, 172	0.05	0.82
Standard length×Lineage×Sex	5.90E-04	1, 172	0.04	0.83
Ascending process height				
Standard length	2.38E-01	1, 172	45.36	<b>&lt;0.001</b>
Lineage	3.36E-02	1, 172	6.42	<b>0.01</b>
Sex	6.41E-03	1, 172	1.22	0.27
Standard length×Lineage	3.47E-02	1, 172	6.62	<b>0.01</b>
Standard length×Sex	6.40E-03	1, 172	1.22	0.27
Lineage×Sex	8.00E-05	1, 172	0.01	0.90
Standard length×Lineage×Sex	1.00E-05	1, 172	0.0002	0.96

Note: The table shows sums of squares (Sum Sq), degrees of freedom (DF), F-values and p-values (bold= $p < 0.05$ ) from Type III ANOVAs with standard length, lineage, and sex as factors.

**TABLE 3** Statistical tests for a comparison of defence traits between wild-caught adult fish from Lake Constance and Lake Geneva.

as no-fish controls (Figure 2g). We measured the standard length of a subset (40 individuals) of each cross type prior to introduction; G×G mean SL=17.09, SE=0.27; hybrid mean SL=15.85, SE=0.28; C×C mean SL=13.65, SE=0.22. Fish were left to forage freely until October 30th (106 days in total), when all remaining individuals were captured and the ponds were drained completely. This provided us with a measure of the total number of surviving fish per pond.

## 2.4 | Morphological analyses

To characterize the external morphology of wild-caught adult fish (180 fish total, 90 of each lineage, 45 of each sex) we removed their internal organs post-mortem (to allow the fixative to better permeate the tissue) and fixed the bodies in a 4% formaldehyde solution for 1 week. We then rinsed these fish in water for 24 h and transferred them to a 30% saturated solution of sodium tetraborate ( $\text{Na}_2[\text{B}_4\text{O}_5(\text{OH})_4] \cdot 8\text{H}_2\text{O}$ ) with 1 g/L of trypsin for 72 h of clearing. Following this, we transferred the fish to a solution of 10 g/L KOH with 80 mg/L of Alizarin red stain for 48 h, and then a solution of 36 mL/L  $\text{H}_2\text{O}_2$  for 4 h. Finally, fish bodies were transferred to glycerol and photographed next to a 2 cm scale bar with hash marks at 1 mm intervals from the dorsal and lateral point of view, with the mouth both open, and closed. We used phenopype software version 2.1.0 (Lürrig, 2021) to digitally landmark photographs of stained fish (Figure S2, Table S1). By converting the number of pixels in the photograph to metric measurements, we were able to calculate linear morphological traits by substituting the x-y coordinates of the

landmarks into the distance formula  $\sqrt{[(x_2-x_1)^2 + (y_2-y_1)^2]}$  (Table S2). From a subset of these landmarks we calculated functional traits that are relevant to foraging such as suction index, displacement advantage, and opercular four-bar linkage using formulae from (McGee et al., 2013; Thompson et al., 2017; Schmid et al., 2019). Together these functional traits quantify the suction force that a fish can generate while feeding, the opening velocity of the lower jaw, and the magnitude of rotation (i.e., speed at which a fish can extend and retract) of the mouth joints. We also measured defensive traits such as dorsal and pelvic spine lengths, and counted the number of bony lateral plates visually. We excluded individuals with damaged mouths or mouthparts ( $n=12$ ) from the analysis of functional traits. At the end of the pond experiment, following capture and euthanasia, we measured the standard length of all juvenile fish by hand with digital callipers to the nearest 0.01 mm, and wet mass to the nearest 0.01 g with a digital microbalance. We then calculated Fulton's condition factor ( $K=100 \times \text{weight}/\text{length}^3$ ) as a measure of individual body condition (Ricker, 1975; Nash et al., 2006). Juveniles were not landmarked in the same fashion as adult fish, and no morphological analyses were performed.

## 2.5 | Microsatellite genotyping, lineage assignment, and survival

As juveniles were introduced into the experiment at a small size (~15 mm SL) we did not tag them with elastomers or transponders to avoid influencing their behaviour or growth. This meant that

**TABLE 4** Mean trait values by population and sex for all morphological measures listed in mm ± SE, with the exception of Displacement Advantage, Suction Index, Opercular 4-bar KT, and Plate Number.

Trait	Constance ♂ (45)	Geneva ♂ (45)	Constance ♀ (45)	Geneva ♀ (45)
Dorsal mouth protrusion	1.83 ± 0.32 (43)	1.68 ± 0.29 (43)	1.35 ± 0.30 (40)	1.36 ± 0.23 (42)
Lower jaw length	4.24 ± 0.45 (43)	4.44 ± 0.34 (43)	3.68 ± 0.44 (40)	3.70 ± 0.39 (42)
Gape width	5.42 ± 0.74 (43)	5.25 ± 0.64 (43)	4.66 ± 0.60 (40)	4.60 ± 0.51 (42)
Displacement Advantage	3.23 ± 0.54 (43)	3.36 ± 0.44 (43)	3.02 ± 0.47 (40)	3.21 ± 0.39 (42)
Suction index	0.0346 ± 0.014 (43)	0.0470 ± 0.026 (43)	0.0468 ± 0.021 (40)	0.0500 ± 0.018 (42)
Opercular 4-bar KT	6.24 ± 1.19 (43)	6.01 ± 1.24 (40)	6.15 ± 1.39 (39)	6.62 ± 1.04 (39)
Standard length	53.7 ± 3.21 (45)	50.3 ± 2.37 (45)	57.0 ± 3.92 (45)	51.2 ± 3.34 (45)
Body width	4.96 ± 0.44 (45)	5.40 ± 0.47 (45)	4.82 ± 0.43 (45)	5.04 ± 0.46 (45)
Dorsal head length	12.6 ± 0.72 (45)	12.3 ± 0.55 (45)	11.7 ± 0.68 (45)	11.3 ± 0.64 (45)
Epaxial height	1.90 ± 0.34 (45)	2.12 ± 0.33 (45)	1.91 ± 0.32 (45)	1.90 ± 0.32 (45)
Eye width	4.79 ± 0.30 (45)	4.72 ± 0.26 (45)	4.54 ± 0.39 (45)	4.46 ± 0.34 (45)
Body depth	12.7 ± 0.78 (45)	13.1 ± 0.62 (45)	14.5 ± 0.98 (45)	13.5 ± 0.84 (45)
Plate number	29.2 ± 5.55 (45)	7.42 ± 2.03 (45)	29.8 ± 1.67 (45)	7.87 ± 4.55 (45)
First dorsal spine length	5.15 ± 0.63 (45)	4.27 ± 0.48 (45)	5.14 ± 0.61 (45)	4.27 ± 0.44 (45)
Second dorsal spine length	5.69 ± 0.61 (45)	4.64 ± 0.44 (45)	5.72 ± 0.71 (45)	4.54 ± 0.41 (45)
Pelvic spine length	8.05 ± 0.85 (45)	6.32 ± 0.75 (45)	8.52 ± 0.93 (45)	6.08 ± 0.74 (45)
Ascending process width	3.08 ± 0.44 (45)	2.88 ± 0.31 (45)	3.37 ± 0.48 (45)	2.91 ± 0.33 (45)
Ascending process height	8.22 ± 0.72 (45)	8.06 ± 0.63 (45)	9.04 ± 0.80 (45)	7.74 ± 0.61 (45)

Note: Numbers in brackets represent the number of individuals measured.

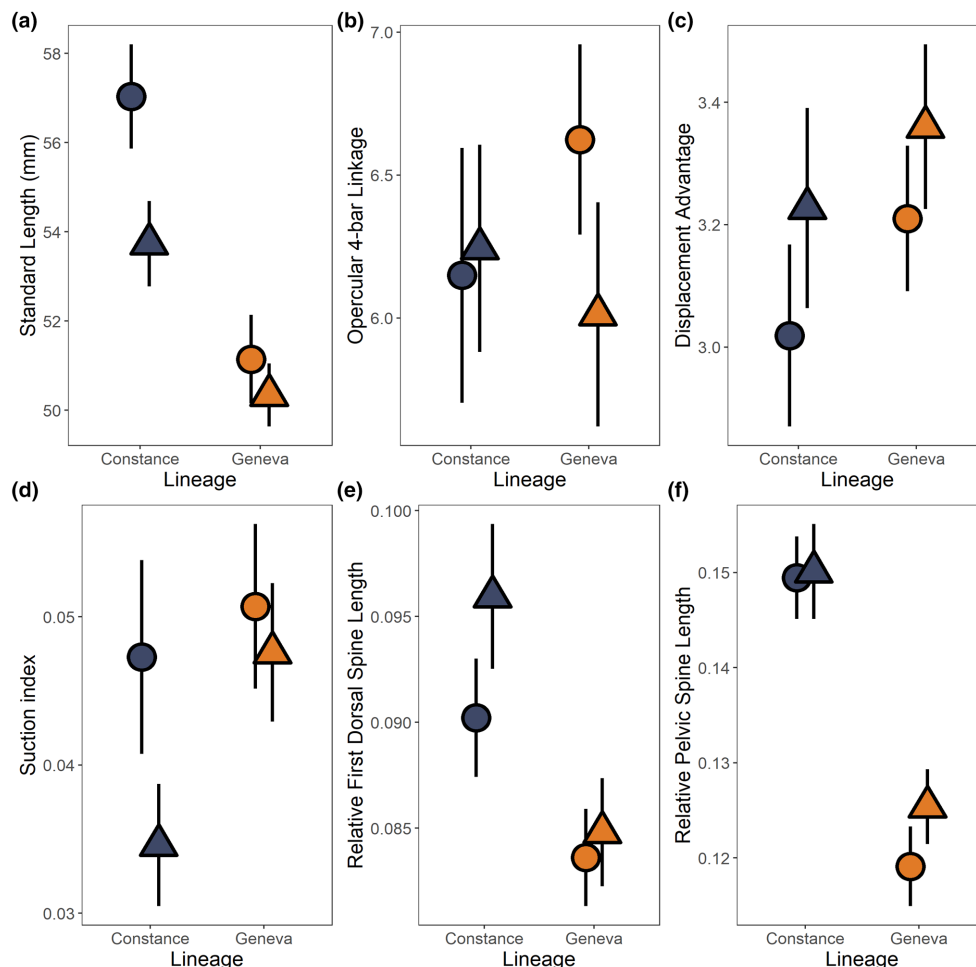


we needed to determine their cross-type after capture to know how many of each lineage survived in each pond. We extracted DNA from juveniles and parents using a HotSHOT protocol that was modified from (Meeker et al., 2007). Following DNA extraction, we identified the lineage that surviving juveniles belonged to as Constance (C×C), Geneva (G×G) or F<sub>1</sub> hybrid (G×C or C×G parents) by genotyping each fish and all parents for ten microsatellite markers (Table S3) (Colosimo et al., 2005; Raeymaekers et al., 2007; Lucek, Sivasundar, et al., 2014) and inferred their lineage using COLONY software version 2.0.6.7 (Jones & Wang, 2010). Microsatellite data were obtained by running a single multiplexed polymerase chain reaction product on an ABI 3130XL and scoring the alleles with GENEMAPPER 4.0 (Applied Biosystems). Of the total 871 recovered juveniles, only six fish could not be assigned to any lineage due to incomplete or erroneous genotype information. These individuals were excluded from our analyses. Once genetic information was available we calculated the selection coefficient for each lineage in each pond as  $S = 1 - (\text{number of surviving juveniles of that lineage} / \text{number surviving of the best-performing$

lineage in that pond). In this way, the lineage with the highest survival in each pond has a score of zero, and larger values indicate the strength of selection against lineages relative to the best performing lineage. We did this to better visualize the differences in survival between lineages and across all ponds, but did not use the survival coefficient in any statistical analyses. See (Best et al., 2017; Brunner et al., 2017) for similar approaches.

## 2.6 | Lipid content analysis

After genotyping and assigning parentage to the experimental fish we randomly selected six individuals of each cross (i.e., G×G, hybrid, and C×C) from each pond (18 individuals per pond; 108 fish in total) for lipid content analysis, as an additional measure of body condition. We extracted lipids from freeze-dried muscle tissue in 2:1 dichloromethane/methanol (4×) and calculated percent lipid by mass by dividing the weight of lipid extract ( $\pm 0.001$  mg) by weight of the freeze-dried tissue ( $\pm 0.001$  mg).



**FIGURE 3** Comparison of morphological traits for wild caught adult female (circles) and male (triangles) fish, of the Constance and Geneva lineages. Points represent the mean value with standard error bars. Relative measures refer to the trait measure, divided by fish standard length. Panel (a) shows a comparison of fish standard length (measured from tip of the snout to end of the last vertebra) between sexes and populations. Panels (b–d) show functional traits that are relevant for fish foraging. Panels (e) and (f) show defensive traits, namely the length of the first dorsal spine, and lateral pelvic spine on the right side of the body.

## 2.7 | Statistical analyses

To test for differences in environmental conditions (e.g., total phosphorus, dissolved organic carbon, etc.) of our pond treatments we performed linear mixed-effect models with treatment (oligotrophic or eutrophic) as a fixed effect, and date as a random effect using the R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). We also tested for differences between prey community composition data (presence/absence) of the treatments using a distance-based redundancy analysis (db-RDA) with the capscale() function in the R package vegan (Oksanen et al., 2020).

To explore the influence of lineage, sex, and their interaction on morphological traits of wild adult stickleback we performed type III ANCOVAs on log transformed morphological variables in R 3.6.1 (R Core Team, 2022) using the car package (Fox & Weisberg, 2019). Since the lineages and sexes differed significantly in standard length we included standard length (also log transformed) and the interactions between it and our other factors in our models as a covariate. For morphological variables relating to the mouth, we used head length in place of standard length.

To identify whether there were differences in survival among genotypes and between treatments we performed a generalized linear mixed model on the proportion surviving (calculated based on our microsatellite genotypes) with treatment, genotype, and their interaction as factors, and pond identity as a random effect. To test for differences in juvenile body condition and lipid content we performed linear mixed-effect models with treatment (oligotrophic or eutrophic) and lineage as fixed effects, and pond as a random effect using the R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017).

## 3 | RESULTS

### 3.1 | Environmental differences between pond treatments

To quantify the effect of eutrophication history on the environmental conditions of our pond treatments we collected data on total phosphorus, dissolved organic carbon, chlorophyll a, zooplankton abundance, and benthic invertebrate abundance. We found that eutrophic ponds had higher levels of total phosphorus, dissolved organic carbon, and chlorophyll a than oligotrophic ponds (Table 5; Figure 2a–c). Oligotrophic ponds had higher zooplankton density, but there was no difference between the treatments in terms of benthic invertebrate abundance (Table 5; Figure 2d,e). With respect to community composition, our db-RDA of prey presence/absence showed divergence in composition ( $F=2.93$ ,  $DF=1$ ,  $p=0.021$ ) between eutrophic and oligotrophic ponds (Figure S15), which was largely explained by the absence of cyclopoid copepods in all the eutrophic ponds, and variation among ponds in the presence/absence of isopods (*Asellus aquaticus*) and caddisfly larvae (*Trichoptera* sp.).

TABLE 5 Linear mixed-effect model results for environmental differences between pond treatments prior to the introduction of juvenile sticklebacks in July 2019.

Predictors	Total phosphorus			Dissolved organic carbon			Chlorophyll a			Macrozooplankton abundance			Benthic invertebrate abundance		
	Estimates	CI	Df	Estimates	CI	Df	Estimates	CI	Df	Estimates	CI	Df	Estimates	CI	Df
(Intercept)	14.57	13.00–16.13	625	6.33	5.62–7.04	625	9.51	8.25–10.77	625	86.59	59.82–113.37	665	547.04	179.92–914.16	22
Treatment (Oligotrophic)	4.86	4.30–5.42	625	0.81	0.64–0.98	625	3.83	3.17–4.50	625	–15.12	–29.20 – –1.05	665	176.38	–190.74 – 543.49	22
<b>Random effects</b>															
$\sigma^2$	50.86		4,64	4.64		76.69	12377.99								
$\tau_{00}$	44.03 <sub>Day</sub>		9.77 <sub>Day</sub>	9.77 <sub>Day</sub>	26.08 <sub>Day</sub>		4051.89 <sub>Day</sub>								
ICC	0.46		0.68	0.68	0.25		0.25								
N	79 <sub>Day</sub>		79 <sub>Day</sub>	79 <sub>Day</sub>	87 <sub>Day</sub>		30 <sub>Day</sub>								
Observations	629		628	628	669		240								
Marginal $R^2$ /Conditional $R^2$	0.199/0.571		0.044/0.692	0.125/0.347	0.014/0.257		0.043/–0.000								

Note: This environmental data was collected over a 3-year period from 2016 to 2019 (see Lurig et al., 2021; Narwani et al., 2019 for details), and highlights the differences in water chemistry and density of the invertebrate prey community.

Bold values indicates statistically significant  $p < 0.05$ .

TABLE 6 Generalized and linear mixed-effect model results for performance measures of pond fish.

	Survival (%)				Body condition (Fulton's K)						
Predictors	Estimates	CI	p	Df	Estimates	CI	p	Df			
(Intercept)	0.57	0.51 to 0.62	<0.001	10.00	1.25	1.23 to 1.26	<0.001	857.00			
Treatment1 (Oligotrophic)	0.01	−0.05 to 0.07	0.700	10.00	−0.01	−0.03 to 0.00	0.153	857.00			
Lineage1 (F <sub>1</sub> Hybrid)	−0.17	−0.21 to −0.13	<0.001	10.00	−0.08	−0.10 to −0.06	<0.001	857.00			
Lineage2 (Geneva)	0.14	0.10 to 0.18	<0.001	10.00	0.02	0.01 to 0.04	0.002	857.00			
Treatment1×Lineage1	0.01	−0.03 to 0.05	0.601	10.00	−0.00	−0.02 to 0.02	0.985	857.00			
Treatment1×Lineage2	−0.01	−0.05 to 0.03	0.721	10.00	−0.00	−0.02 to 0.01	0.784	857.00			
Random effects											
σ <sup>2</sup>			0.00			0.03					
τ <sub>00</sub>			0.00 <sub>Pond</sub>			0.00 <sub>Pond</sub>					
ICC			0.51			0.01					
N			6 <sub>Pond</sub>			6 <sub>Pond</sub>					
Observations			18			865					
Marginal R <sup>2</sup> /Conditional R <sup>2</sup>			0.706/0.857			0.100/0.105					
Pairwise	Estimate	SE	df	t	p	Estimate	SE	df	t	p	Estimate
Constance – F <sub>1</sub> Hybrid	−0.312	0.0348	8	−8.971	<0.0001	−0.1052	0.0143	857	−7.343	<0.0001	−2.000
Constance – Geneva	−0.202	0.0348	8	−5.813	0.0010	−0.1388	0.0148	859	−9.370	<0.0001	−1.785
F <sub>1</sub> Hybrid – Geneva	0.110	0.0348	8	3.158	0.0322	−0.0337	0.0127	857	−2.662	0.0215	0.215

Note: Data on the proportion of each genotype surviving was analysed with a GLMM, while other performance traits were analysed with LMMs. Models below are comparing the effect of treatment and fish lineage on various performance measures with pond identity as a random effect. The lower section of this table displays pairwise post-hoc tests between the three fish lineages. Values highlighted in bold represent  $p < 0.05$ .

### 3.2 | Morphological differences between wild caught adults

Adult fish of the two lineages differed significantly in several morphological traits (see Tables 1–4 and Figures S3–S14 for a complete list), but here we focus specifically on those that may have functional relevance for foraging and antipredator defence. Firstly, fish of both sexes from the Constance lineage were larger (Figure 3a) than those from Geneva. With respect to functional mouth traits, there was a significant interaction between lineage and sex for opercular four-bar linkage (Figure 3b, Table 1) indicating females of the Geneva lineage can generate more force when opening their jaws than either sex from the Constance lineage, while the opposite is true for Geneva males. For displacement advantage, there was a significant effect of sex and lineage, suggesting that male fish, and fish from the Geneva lineage can open their jaws more rapidly (Figure 3c, Table 1). Mouth protrusion also showed a significant three-way interaction (Table 1), and gape width was marginally significant ( $p < 0.054$ ) with males of both lineage having wider mouths that protrude further, with greater dimorphism in the Constance lineage (Table 4). Finally, there was a significant interaction between sex and lineage for suction index, suggesting that females do not differ between lineages in the amount of suction force they can generate, while males do, with Geneva males being capable of generating greater suction force (Figure 3d, Table 1).

The Constance and Geneva lineages differed markedly in defence traits, with Constance fish having more bony lateral plates (Figure S11, Table 4), and longer dorsal and pelvic spines (Figure 3e,f, Table 3). The width of the ascending process was most strongly predicted by standard length (but not lineage), while there was an interactive effect between sex and lineage on ascending process height such that Constance females had significantly taller ascending processes (Figure S14).

### 3.3 | Body condition, lipid content, and survival of juveniles in the pond experiment

We used four informative measures of individual performance: Fulton's K (a body condition index), percent lipid content from muscle, fish standard length (mm), and fish mass (g). Our linear mixed-effect model of fish body condition showed a significant effect of lineage but not of the environmental contrast (Table 6). Pairwise post-hoc tests showed that fish from the Constance lineage were in significantly lower body condition than F<sub>1</sub> hybrids and Geneva fish, and Geneva fish were in better condition than F<sub>1</sub> hybrids (Table 6, Figure 4). Our linear mixed-effect model of fish lipid content showed a significant effect of lineage but not of the environmental conditions (Table 6). Pairwise post-hoc tests showed that fish from the Constance lineage had significantly lower lipid content than F<sub>1</sub>

Lipid content (% by mass)					Standard length (mm)				Mass (g)					
Estimates	CI		p	Df	Estimates	CI		p	Df	Estimates	CI		p	Df
13.02	11.48 to 14.56		<0.001	99.00	22.74	21.87 to 23.62		<0.001	857.00	0.15	0.13 to 0.17		<0.001	857.00
-0.23	-1.77 to 1.31		0.769	99.00	0.09	-0.78 to 0.97		0.838	857.00	0.00	-0.02 to 0.02		0.989	857.00
-1.26	-2.20 to -0.33		0.008	99.00	-1.08	-1.34 to -0.82		<0.001	857.00	-0.03	-0.04 to -0.03		<0.001	857.00
0.74	-0.16 to 1.64		0.107	99.00	1.20	0.97 to 1.42		<0.001	857.00	0.03	0.02 to 0.03		<0.001	857.00
-0.02	-0.95 to 0.92		0.973	99.00	-0.24	-0.50 to 0.02		0.070	857.00	-0.00	-0.01 to 0.00		0.218	857.00
0.18	-0.72 to 1.08		0.692	99.00	0.21	-0.01 to 0.44		0.063	857.00	0.01	-0.00 to 0.01		0.061	857.00
11.33					6.15					0.00				
3.06 <sub>Pond</sub>					1.15 <sub>Pond</sub>					0.00 <sub>Pond</sub>				
0.21					0.16					0.11				
6 <sub>Pond</sub>					6 <sub>Pond</sub>					6 <sub>Pond</sub>				
107					865					865				
0.057/0.258					0.108/0.248					0.107/0.209				
SE	df	t	p	Estimate	SE	df	t	p	Estimate	SE	df	t	p	
0.812	97.5	-2.462	0.0409	-2.277	0.219	855	-10.403	<0.0001	-0.0611	0.00578	855	-10.565	<0.0001	
0.824	97.2	-2.165	0.0824	-0.965	0.226	855	-4.259	0.0001	-0.0363	0.00598	856	-6.073	<0.0001	
0.794	98.3	0.271	0.9605	1.313	0.193	855	6.788	<0.0001	0.0248	0.00511	855	4.847	<0.0001	

hybrids, but were not different from Geneva fish. There was no difference in mean lipid content between Geneva and  $F_1$  hybrids. In the case of survival, our generalized linear mixed model showed a significant effect of genotype but not of treatment (Table 6), with Constance fish experiencing the lowest survival rates and  $F_1$  hybrids the highest (Figure 5). Across all ponds, mean survival was lowest for Constance fish ( $39.4\% \pm 3.98$  SE), intermediate for both Geneva fish ( $59.6\% \pm 1.77$  SE) and highest for  $F_1$  hybrids ( $70.6\% \pm 3.45$  SE).

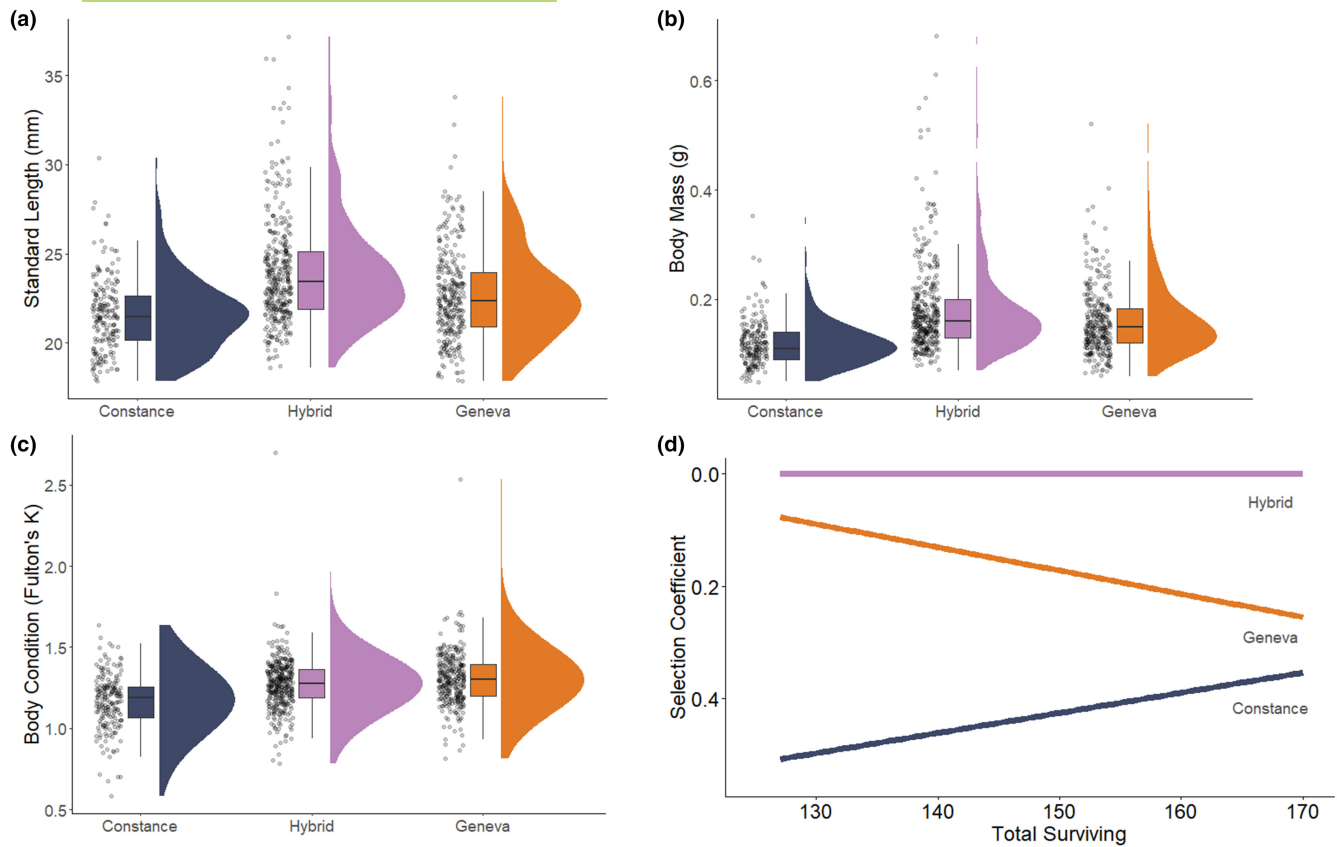
## 4 | DISCUSSION

The colonization of Switzerland by threespine stickleback is a complicated mixture of natural range expansion, anthropogenic introductions from multiple source populations, and secondary contact between (at least) three ecologically divergent lineages (Hudson, Lucek, et al., 2021). We know that secondary contact throughout the Swiss plateau is occurring, and that hybridization is a common feature of the landscape (Lucek et al., 2010; Lucek, Lemoine, & Seehausen, 2014; Roy et al., 2015; Lucek, 2016; Marques et al., 2016; Marques, Lucek, et al., 2019), but what remains unclear is how introgression among lineages will influence the spread or dynamics of the ongoing invasion. Since the two lineages in this study have had a different history of adaptation to freshwater, and are ecologically consistent with how they use their habitat (e.g., Geneva lineage is more benthic, Constance lineage more pelagic) we expect

this to influence both their morphology, and fitness in different freshwater habitats.

From our investigation of wild adult fish, we found that the two lineages showed differences in functional mouthpart traits (e.g., displacement advantage, suction index, and opercular four-bar linkage) that were somewhat in line with our expectations based on niche use, though not as extreme as classical benthic-limnetic stickleback sympatric species pairs (McGee et al., 2013). For example, the higher suction index values in Geneva fish suggest heightened performance in feeding on attached macroinvertebrates, while Constance fish did not show significantly higher displacement advantage that would be expected from a planktivorous limnetic ecotype (Schmid et al., 2019). It is important to remember that although we treat these two lineages as “benthic-like” (Geneva) and “limnetic-like” (Constance) lake ecotypes, they arise from independent colonizations in two different watersheds and do not reflect a natural case of ecotype divergence. As such, there may be overlap in the niche use and diet composition of these lineages (since they are not directly in competition for the vast majority of their ranges). In our study, the lineages did show sexual dimorphism in some functional traits that could suggest sexual divergence in resource use (McGee & Wainwright, 2013), which could have implications for hybrid performance in different habitats. For example, previous work has shown that ecotypes differing in functional traits can exert strong effects on their ecosystems, altering trophic structure and community composition (Harmon et al., 2009; Matthews





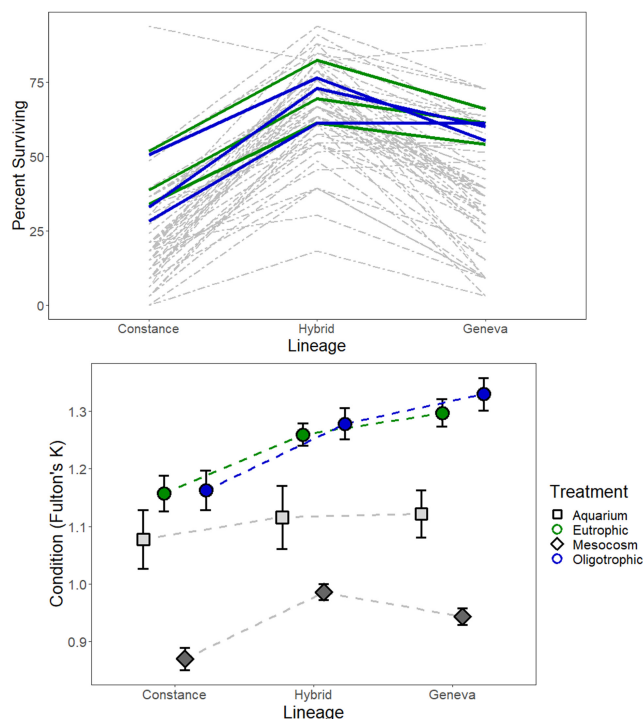
**FIGURE 4** In all panels, fish from the Lake Constance lineage are represented in navy, hybrids in violet, and fish from the Lake Geneva lineage are in orange. Panels (a–c) show raincloud plots for fish standard length (mm), body mass (g), and body condition (Fulton's K) by lineage for all ponds. Dots represent individual fish, box plots show the mean with standard error bars, and histograms show the trait distribution. Panel (d) shows the strength of selection by lineage in relation to the total number of surviving juveniles. This was generated using the selection coefficient that we calculated based on the surviving fish collected at the end of the experiment. Here, the selection coefficient for a given lineage in a single pond is calculated as  $S = 1 - (\text{number of surviving juveniles of that lineage} / \text{number surviving of the best performing lineage in that pond})$ , such that the best performing lineage will have a value of 0. Across all ponds in this experiment, hybrids experienced the highest survival, hence in each pond  $S = 0$  for hybrids. The survival of Constance and Geneva fish differed, depending on the total number of fish surviving in a given treatment, thus, the slope of the lines (i.e., the strength of selection) depended on the total number of fish present at the end of the experiment.

et al., 2016; Best et al., 2017). Furthermore, with a more recent marine ancestry the defensive morphology of Constance lineage fish (i.e., long spines, bony lateral plates, Figure 3, Figure S11) may weakened predation pressure in the “marine-like” open water habitat of a large freshwater lake, such as Lake Constance. Geneva fish, on the other hand show the typical reduction in plate number and spine length (Figure 3, Figure S11) that is observed in stickleback populations during freshwater colonization (Foster & Bell 1994; McKinnon & Rundle 2002), thus they are likely more vulnerable to predation from piscivores, and might be more restricted to habitats with macrophytes as refuge.

In our pond experiment, we found that fish from the Geneva lineage, and  $F_1$  hybrids consistently outperformed fish from the Constance lineage regardless of the environmental context (eutrophication history) that they experienced. Such environment-independent differences in the rank order of survival were consistent with a comparable study performed in mesocosms and aquaria using the same lineage combinations (Best et al., 2017)

(Figure 5). Considering these two studies together, across a broad range of scales (aquaria, mesocosms, and ponds) we see consistent, environment-independent fitness differences between these introduced lineages, with Constance fish performing poorly in a wide range of experimental conditions. The elevated performance of  $F_1$  hybrids over Constance lineage fish, and in some cases (e.g., survival) Geneva lineage fish, suggests that heterosis occurs when these lineages hybridize. However, as we only compared  $F_1$  hybrids to parental lineages, we can only speculate on the effect of continued hybridization in subsequent generations. The effect of heterosis in natural populations, while common, is often short-lived, with collapses in hybrid fitness occurring as backcrossing becomes more frequent (Emmrich et al., 2015). Nevertheless, the production of  $F_1$  hybrids between these lineages may facilitate spread, as seen in other systems (Rius & Darling, 2014; Hahn & Rieseberg, 2017; Shi et al., 2018).

Hybrid individuals are often intermediate in morphology and performance (Hatfield & Schluter, 1999; Rundle, 2002; Gow et al., 2007;



**FIGURE 5** Top panel – the percentage of surviving fish by lineage for each pond from this experiment (solid lines; green=eutrophic, blue=oligotrophic) and the same lineages and their hybrids when raised in mesocosms from (Best et al., 2017) (dashed grey lines). Bottom panel – a comparison of mean body condition (Fulton's K) for each lineage at different scales (fish reared in aquaria, mesocosms, and ponds) between the two studies. Coloured circles represent fish from this study, while grey squares and diamonds are data from (Best et al., 2017).

Arnegard et al., 2014), though recent work has shown that in many cases they can demonstrate a parental bias (i.e.,  $F_1$  offspring resemble one parent more than the other) (Thompson et al., 2020). Such bias can be beneficial in cases where a contact zone spans a variety of environmental conditions or habitat types, as hybrids can persist in the habitat of the parent that they resemble, or even in novel habitats that are occupied by neither parent when transgressive phenotypes are produced (Abbott et al., 2010). From our experimental populations, it is clear that the performance of  $F_1$  hybrid fish was more similar to that of Geneva lineage fish than Constance lineage fish (Figures 4 and 5). Though we have documented morphological differences between adult fish of these lineages, how this translates into differences in juvenile growth and survival is poorly known. From a recent experimental study, we know that lab-reared populations of these lineages and their hybrids differ in foraging performance on a mixed community of invertebrate prey, and that this performance variation can be attributed to a combination of morphological and behavioural differences acting in concert (Moosmann et al., 2023). Previous work has found that morphological intermediacy in second generation hybrids can lead to reduced fitness (Arnegard et al., 2014) but we did not see this in our current experiment. From this, we can conclude that further empirical work

confirming the link between phenotypic patterns and performance across a range of environmental conditions could shed light on the fitness of hybrid individuals between these lineages.

The environments of our pond treatments differed in prey community composition and productivity at the outset of the experiment, yet despite these differences,  $F_1$  hybrid fish exhibited higher fitness over parental lineage fish in both eutrophic and oligotrophic conditions. Previous experiments comparing divergent stickleback ecotypes (benthic-limnetic or lake-stream) in field enclosures have typically found that  $F_1$  hybrids have reduced (Hatfield & Schluter, 1999; Vamossi et al., 2000) or intermediate (Schluter, 1995; Moser et al., 2015) fitness compared to their parental lineages, though in some case  $F_2$  backcrosses can exhibit elevated fitness (Rundle, 2002). In our experiment, it is possible that the environmental context did not provide an opportunity for the two lineages to exhibit differential performance. Though the environmental conditions in our ponds did vary (Figure 2, Table 1), it is likely that pond conditions overall were more similar to a shallow littoral habitat, perhaps more akin to the niche inhabited by Geneva fish. Perhaps this explains why the Constance lineage fish consistently perform poorly in semi-natural habitats such as ponds or mesocosms. To experimentally simulate the pelagic environment in which Constance stickleback are found is extremely challenging and would either require whole-lake experimentation, or reciprocal transplant enclosure experiments as has been done for benthic and limnetic species pairs (Hatfield & Schluter, 1999; Vamossi et al., 2000; Rundle, 2002). Although, even in such experiments the limnetic species does not outperform the benthic species, but the performance is rather equalized (Hatfield & Schluter, 1999; Rundle, 2002).

Threespine stickleback are hyperabundant in Lake Constance (Alexander et al., 2016), and reach exceptionally large body sizes compared to other European freshwater populations (Hudson, Lucek, et al., 2021), so why do they consistently perform poorly under a broad range of experimental conditions (i.e., in the lab), mesocosms, and experimental ponds? Compared to marine ecosystems, food quality is typically lower in freshwater, particularly with respect to omega-3 long chain fatty acids (Twining et al., 2021). For a marine fish species that has colonized freshwater, this shift in the nutritional environment could impose strong selection on both ecological traits (e.g., dietary preferences, habitat use, and morphology) and metabolic traits (e.g., in vivo synthesis of fatty acids). Previous studies have shown that freshwater stickleback lineages, including those in this study, differ in copy number for a gene (*FADS2*) that is responsible for production of an enzyme that catalyses desaturation in DHA biosynthesis (Ishikawa et al., 2019). Copy number variation in this gene has arisen during the transition from marine to freshwater, but also during ecological divergence (e.g., lake-stream ecotype formation) within freshwater (Ishikawa et al., 2021). In a previous lab experiment, (Hudson, Ladd, et al., 2021) reared these lineages and their hybrids on the same diet, and found that Geneva fish can endogenously produce more DHA than Constance fish. Given that our experimental treatments are likely lower in food quality than a natural lake ecosystem (i.e., predominantly benthic invertebrates, and

low plankton abundance) it is possible that Constance lineage fish were experiencing DHA deficiencies, leading to reduced growth and fitness as a result. In the former study, hybrid and backcross individuals produced more DHA than Constance lineage fish (especially males) (Hudson, Ladd, et al., 2021), and thus the additional *FADS2* copies contributed by Geneva lineage parents may help prevent DHA deficiency in this environment. Finally, the fitness differences observed between lineages may simply be the result of life-history variation in growth and time to maturity.

Recombination of genetic variants through hybridization can lead to rapid adaptation (Hedrick, 2013; Keller et al., 2013; Marques, Meier, & Seehausen, 2019), but the specific outcomes depend on the fitness of hybrids in the environmentally relevant conditions in nature. On the one hand, hybridization can introduce alleles (or coadapted gene complexes; Burton et al., 1999) into a population that can serve as a source of variation for selection to novel environmental conditions (Lewontin & Birch, 1966). Previous work has shown that introductions derived from multiple source populations can lead to increased genetic diversity and allow for invasive hybrid swarms to spread across a wide range of environmental conditions (Lavergne & Molofsky, 2007; Dlugosch & Parker, 2008; van Boheemen et al., 2017). On the other hand, hybrids may have enhanced fitness over their parental lineages, which is defined as heterosis, but the extent of such fitness differences can also be strongly environment dependent (Arnold et al., 2012). There is considerable work on the environmental dependence and independence of heterosis in natural populations (Hahn & Rieseberg, 2017; Wagner et al., 2021; Thompson & Schluter, 2022; Thompson et al., 2022). However, the benefits of heterosis are often transient in sexually reproducing organisms (Lee, 2002), and may not predict longer term evolutionary outcomes in natural populations (Wei & Zhang, 2018; Harkness et al., 2019).

## 5 | FUTURE DIRECTIONS AND CONCLUSIONS

Knowing that there is the potential for heterosis among divergent European stickleback lineages, and that this phenomenon can influence invasiveness, what might we anticipate for the outcomes of secondary contact between them? In the case of benthic-limnetic stickleback species pairs, heterosis occurs but its effects are counteracted by environment-dependent hybrid breakdown, with the net outcome being ecological speciation (Thompson & Schluter, 2022). In the Swiss system however, these populations are not entirely sympatric, but rather span a ~300 Km zone of secondary contact. Thus, we expect that adaptive introgression will be the more likely scenario, with genetic contributions from each lineage driving the colonization of different habitats across pre-alpine lake ecosystems. Based on the niche use of these lineages in the wild, we might speculate that Eastern European alleles will promote the colonization of pelagic habitats, while Western European alleles could facilitate

colonization of streams and littoral habitats. Previous work has already provided some evidence of this occurring. For example, differentiation between lake and stream ecotypes in Lake Constance is the result of introgression from Western European alleles across the hybrid zone (Marques, Lucek, et al., 2019). In this case, hybridization has promoted colonization of stream habitats and rapid divergence between lake and stream ecotypes. Two other studies in the same region (Lucek, Lemoine, & Seehausen, 2014; Lucek, 2016) have documented the spread of the fully plated *Eda<sub>c</sub>* allele, and demonstrated that introgression from the Eastern European lineage has facilitated range expansion into the pelagic zone of Lake Geneva. Thus, bidirectional gene flow and adaptive introgression appears to have aided the Eastern lineage in stream colonization, and the Western lineage in lake colonization. From this work and previous experiments (Best et al., 2017), we see consistent evidence for *F<sub>1</sub>* hybrids outperforming Constance and Geneva lineage fish. However, as we have yet to experimentally establish an ecosystem context where Constance fish outperform Geneva fish, we are uncertain how the outcome of secondary contact will play out in natural populations.

## AUTHOR CONTRIBUTIONS

**Cameron Marshall Hudson:** Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); resources (equal); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Maria Cuenca Cambronero:** Conceptualization (equal); methodology (equal); resources (equal); validation (equal); writing – review and editing (equal). **Marvin Moosmann:** Conceptualization (equal); methodology (equal); resources (equal); visualization (equal); writing – review and editing (equal). **Anita Narwani:** Conceptualization (equal); methodology (equal); resources (equal); writing – review and editing (equal). **Piet Spaak:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); writing – review and editing (equal). **Ole Seehausen:** Conceptualization (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (equal). **Blake Matthews:** Conceptualization (equal); funding acquisition (equal); methodology (equal); project administration (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

Data is available at Dryad <https://doi.org/10.5061/dryad.dr7sqvb33>.

## ORCID

Cameron Marshall Hudson  <https://orcid.org/0000-0003-3298-4510>

## REFERENCES

- Abbott, R. J., Hegarty, M. J., Hiscock, S. J., & Brennan, A. C. (2010). Homoploid hybrid speciation in action. *Taxon*, 59, 1375–1386.
- Alexander, T., & Seehausen, O. (2021). *Diversity, distribution and community composition of fish in perialpine lakes*. Das Wasserforschungsinstitut des ETH-Bereichs.
- Alexander, T. J., Vonlanthen, P., Périat, G., Raymond, J., Degiorgi, F., & Seehausen, O. (2016). Artenvielfalt und Zusammensetzung der Fischpopulation im Bodensee. *Projet Lac*, Eawag. Google Scholar.
- Arnegard, M. E., McGee, M. D., Matthews, B., Marchinko, K. B., Conte, G. L., Kabir, S., Bedford, N., Bergek, S., Chan, Y. F., Jones, F. C., Kingsley, D. M., Peichel, C. L., & Schluter, D. (2014). Genetics of ecological divergence during speciation. *Nature*, 511, 307–311.
- Arnold, M. L., Ballerini, E. S., & Brothers, A. N. (2012). Hybrid fitness, adaptation and evolutionary diversification: Lessons learned from Louisiana irises. *Heredity*, 108, 159–166.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Berner, D., Roesti, M., Hendry, A. P., & Salzburger, W. (2010). Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Molecular Ecology*, 19, 4963–4978.
- Best, R. J., Anaya-Rojas, J. M., Leal, M. C., Schmid, D. W., Seehausen, O., & Matthews, B. (2017). Transgenerational selection driven by divergent ecological impacts of hybridizing lineages. *Nature Ecology and Evolution*, 1, 1757–1765.
- Birchler, J. A., Yao, H., Chudalayandi, S., Vaiman, D., & Veitia, R. A. (2010). Heterosis. *The Plant Cell*, 22, 2105–2112.
- Bretzel, J. B., Geist, J., Guegle, S. M., Baer, J., & Brinker, A. (2021). Feeding ecology of invasive three-spined stickleback (*Gasterosteus aculeatus*) in relation to native juvenile Eurasian perch (*Perca fluviatilis*) in the pelagic zone of upper Lake Constance. *Frontiers of Environmental Science & Engineering in China*, 9, 254.
- Brunner, F. S., Anaya-Rojas, J. M., Matthews, B., & Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 3678–3683.
- Burton, R. S., Rawson, P. D., & Edmands, S. (1999). Genetic architecture of physiological phenotypes: Empirical evidence for Coadapted gene Complexes1. *American Zoologist*, 39, 451–462.
- Chen, Z. J. (2013). Genomic and epigenetic insights into the molecular bases of heterosis. *Nature Reviews. Genetics*, 14, 471–482.
- Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Jr., Dickson, M., Grimwood, J., Schmutz, J., Myers, R. M., Schluter, D., & Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science*, 307, 1928–1933.
- Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer Associates.
- Dean, L. L., Magalhaes, I. S., Foote, A., D'Agostino, D., McGowan, S., & MacColl, A. D. C. (2019). Admixture between ancient lineages, selection, and the formation of sympatric stickleback species-pairs. *Molecular Biology and Evolution*, 36, 2481–2497.
- Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17, 431–449.
- Drake, J. M. (2006). Heterosis, the catapult effect and establishment success of a colonizing bird. *Biology Letters*, 2, 304–307.
- Eckmann, R., & Engesser, B. (2019). Reconstructing the build-up of a pelagic stickleback (*Gasterosteus aculeatus*) population using hydro-acoustics. *Fisheries Research*, 210, 189–192.
- Ellstrand, N. C., & Schierenbeck, K. A. (2006). Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica*, 148, 35–46.
- Emmrich, P. M. F., Roberts, H. E., & Pancaldi, V. (2015). A Boolean gene regulatory model of heterosis and speciation. *BMC Evolutionary Biology*, 15, 24.
- Epifanio, J., & Philipp, D. (2000). Simulating the extinction of parental lineages from introgressive hybridization: The effects of fitness, initial proportions of parental taxa, and mate choice. *Reviews in Fish Biology and Fisheries*, 10, 339–354.
- Fang, B., Merilä, J., Matschiner, M., & Momigliano, P. (2020). Estimating uncertainty in divergence times among three-spined stickleback clades using the multispecies coalescent. *Molecular Phylogenetics and Evolution*, 142, 106646.
- Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M., & Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. *Molecular Phylogenetics and Evolution*, 127, 613–625.
- Fiévet, J. B., Nidelet, T., Dillmann, C., & de Vienne, D. (2018). Heterosis is a systemic property emerging from non-linear genotype-phenotype relationships: Evidence from in vitro genetics and computer simulations. *Frontiers in Genetics*, 9, 159.
- Foster, S. A., & Bell, M. A. (1994). *The evolutionary biology of the threespine stickleback*. Oxford University Press.
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (Third ed.). Sage <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Gow, J. L., Peichel, C. L., & Taylor, E. B. (2007). Ecological selection against hybrids in natural populations of sympatric threespine sticklebacks. *Journal of Evolutionary Biology*, 20, 2173–2180.
- Hahn, M. A., & Rieseberg, L. H. (2017). Genetic admixture and heterosis may enhance the invasiveness of common ragweed. *Evolutionary Applications*, 10, 241–250.
- Hanson, D., Moore, J.-S., Taylor, E. B., Barrett, R. D. H., & Hendry, A. P. (2016). Assessing reproductive isolation using a contact zone between parapatric lake-stream stickleback ecotypes. *Journal of Evolutionary Biology*, 29, 2491–2501.
- Harkness, A., Brandvain, Y., & Goldberg, E. E. (2019). The evolutionary response of mating system to heterosis. *Journal of Evolutionary Biology*, 32, 476–490.
- Harmon, L. J., Matthews, B., Des Roches, S., Chase, J. M., Shurin, J. B., & Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. *Nature*, 2009, 1167–1170.



- Hatfield, T., & Schluter, D. (1999). Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. *Evolution*, 53, 866–873.
- Hedrick, P. W. (2013). Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*, 22, 4606–4618.
- Hendry, A. P., Bolnick, D. I., Berner, D., & Peichel, C. L. (2009). Along the speciation continuum in sticklebacks. *Journal of Fish Biology*, 75, 2000–2036.
- Hill, J. K., Griffiths, H. M., & Thomas, C. D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annual Review of Entomology*, 56, 143–159.
- Hochholdinger, F., & Hoecker, N. (2007). Towards the molecular basis of heterosis. *Trends in Plant Science*, 12, 427–432.
- Hudson, C. M., Ladd, S. N., Leal, M. C., Schubert, C. J., Seehausen, O., & Matthews, B. (2022). Fit and fatty freshwater fish: contrasting polyunsaturated fatty acid phenotypes between hybridizing stickleback lineages. *Oikos*, 2022. <https://doi.org/10.1111/oik.08558>
- Hudson, C. M., Lucek, K., Marques, D. A., Alexander, T. J., Moosmann, M., Spaak, P., Seehausen, O., & Matthews, B. (2021). Threespine stickleback in Lake Constance: The ecology and genomic substrate of a recent invasion. *Frontiers in Ecology and Evolution*, 8, 529.
- Hudson, C. M., Vidal-García, M., Murray, T. G., & Shine, R. (2020). The accelerating anuran: Evolution of locomotor performance in cane toads (*Rhinella marina*, Bufonidae) at an invasion front. *Proceedings of the Biological Sciences*, 287, 20201964.
- Irwin, D. E., Bensch, S., & Price, T. D. (2001). Speciation in a ring. *Nature*, 409, 333–337.
- Ishikawa, A., Kabeya, N., Ikeya, K., Kakioka, R., Cech, J. N., Osada, N., Leal, M. C., Inoue, J., Kume, M., Toyoda, A., Tezuka, A., Nagano, A. J., Yamasaki, Y. Y., Suzuki, Y., Kokita, T., Takahashi, H., Lucek, K., Marques, D., Takehana, Y., ... Kitano, J. (2019). A key metabolic gene for recurrent freshwater colonization and radiation in fishes. *Science*, 364, 886–889.
- Ishikawa, A., Stuart, Y. E., Bolnick, D. I., & Kitano, J. (2021). Copy number variation of a fatty acid desaturase gene *Fads2* associated with ecological divergence in freshwater stickleback populations. *Biology Letters*, 17, 20210204.
- Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10, 551–555.
- Keagy, J., Lettieri, L., & Boughman, J. W. (2016). Male competition fitness landscapes predict both forward and reverse speciation. *Ecology Letters*, 19, 71–80.
- Keller, I., Wagner, C. E., Greuter, L., Mwaiko, S., Selz, O. M., Sivasundar, A., Wittwer, S., & Seehausen, O. (2013). Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Molecular Ecology*, 22, 2848–2863.
- Kitano, J., Mori, S., & Peichel, C. L. (2007). Phenotypic divergence and reproductive isolation between sympatric forms of Japanese threespine sticklebacks. *Biological Journal of the Linnean Society of London*, 91, 671–685.
- Kolbe, J. J., Glor, R. E., Rodríguez Schettino, L., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431, 177–181.
- Kottelat, M., & Freyhof, J. (2007). *Handbook of European freshwater fishes* (646). Publications Kottelat.
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>
- Labroo, M. R., Studer, A. J., & Rutkoski, J. E. (2021). Heterosis and hybrid crop breeding: A multidisciplinary review. *Frontiers in Genetics*, 12, 643761.
- Lackey, A. C. R., & Boughman, J. W. (2017). Evolution of reproductive isolation in stickleback fish. *Evolution*, 71, 357–372.
- Lavergne, S., & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 3883–3888.
- Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, 17, 386–391.
- Lewontin, R. C., & Birch, L. C. (1966). Hybridization AS a source of variation for adaptation to new environments. *Evolution*, 20, 315–336.
- Lippman, Z. B., & Zamir, D. (2007). Heterosis: Revisiting the magic. *Trends in Genetics*, 23, 60–66.
- Lucek, K. (2016). Cryptic invasion drives phenotypic changes in central European threespine stickleback. *Conservation Genetics*, 17, 993–999.
- Lucek, K., Lemoine, M., & Seehausen, O. (2014). Contemporary ecotypic divergence during a recent range expansion was facilitated by adaptive introgression. *Journal of Evolutionary Biology*, 27, 2233–2248.
- Lucek, K., Roy, D., Bezault, E., Sivasundar, A., & Seehausen, O. (2010). Hybridization between distant lineages increases adaptive variation during a biological invasion: Stickleback in Switzerland. *Molecular Ecology*, 19, 3995–4011.
- Lucek, K., Sivasundar, A., Kristjánsson, B. K., Skúlason, S., & Seehausen, O. (2014). Quick divergence but slow convergence during ecotype formation in lake and stream stickleback pairs of variable age. *Journal of Evolutionary Biology*, 27, 1878–1892.
- Lucek, K., Sivasundar, A., Roy, D., & Seehausen, O. (2013). Repeated and predictable patterns of ecotypic differentiation during a biological invasion: Lake-stream divergence in parapatric Swiss stickleback. *Journal of Evolutionary Biology*, 26, 2691–2709.
- Lürig, M. D. (2021). phenopype: A phenotyping pipeline for Python. *Methods in Ecology and Evolution*. <https://doi.org/10.1111/2041-210x.13771>
- Lürig, M. D., Narwani, A., Penson, H., Wehrli, B., Spaak, P., & Matthews, B. (2021). Non-additive effects of foundation species determine the response of aquatic ecosystems to nutrient perturbation. *Ecology*, 102, e03371.
- MacPherson, A., Wang, S., Yamaguchi, R., Rieseberg, L. H., & Otto, S. P. (2022). Parental population range expansion before secondary contact promotes Heterosis. *The American Naturalist*, 200, E000–E015.
- Mäkinen, H. S., Cano, J. M., & Merilä, J. (2006). Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Molecular Ecology*, 15, 1519–1534.
- Mäkinen, H. S., & Merilä, J. (2008). Mitochondrial DNA phylogeography of the three-spined stickleback (*Gasterosteus aculeatus*) in Europe—Evidence for multiple glacial refugia. *Molecular Phylogenetics and Evolution*, 46, 167–182.
- Marques, D. A., Lucek, K., Meier, J. I., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2016). Genomics of rapid incipient speciation in sympatric Threespine stickleback. *PLoS Genetics*, 12, e1005887.
- Marques, D. A., Lucek, K., Sousa, V. C., Excoffier, L., & Seehausen, O. (2019). Admixture between old lineages facilitated contemporary ecological speciation in Lake Constance stickleback. *Nature Communications*, 10, 4240.
- Marques, D. A., Meier, J. I., & Seehausen, O. (2019). A combinatorial view on speciation and adaptive radiation. *Trends in Ecology & Evolution*, 34, 531–544.
- Matthews, B., Aebischer, T., Sullam, K. E., Lundsgaard-Hansen, B., & Seehausen, O. (2016). Experimental evidence of an eco-evolutionary feedback during adaptive divergence. *Current Biology*, 26, 483–489.

- McGee, M. D., Schluter, D., & Wainwright, P. C. (2013). Functional basis of ecological divergence in sympatric stickleback. *BMC Evolutionary Biology*, 13, 277.
- McGee, M. D., & Wainwright, P. C. (2013). Sexual dimorphism in the feeding mechanism of threespine stickleback. *The Journal of Experimental Biology*, 216, 835–840.
- McKinnon, J. S., & Rundle, H. D. (2002). Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution*, 17, 480–488.
- Meeker, N. D., Hutchinson, S. A., Ho, L., & Trede, N. S. (2007). Method for isolation of PCR-ready genomic DNA from zebrafish tissues. *BioTechniques*, 43, 610, 612, 614.
- Moody, M. L., & Les, D. H. (2002). Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proceedings of the National Academy of Sciences of the United States of America*, 2002, 14867–14871.
- Moosmann, M., Hudson, C. M., Seehausen, O., & Matthews, B. (2023). The phenotypic determinants of diet variation between divergent lineages of threespine stickleback. *Evolution*, 77, 13–25.
- Moser, D., Kueng, B., & Berner, D. (2015). Lake-stream divergence in stickleback life history: A plastic response to trophic niche differentiation? *Evolutionary Biology*, 42, 328–338.
- Narwani, A., Reyes, M., Pereira, A. L., Penson, H., Dennis, S. R., Derrer, S., Spaak, P., & Matthews, B. (2019). Interactive effects of foundation species on ecosystem functioning and stability in response to disturbance. *Proceedings of the Biological Sciences*, 286, 20191857.
- Nash, R., Valencia, A., & Geffen, A. (2006). The origin of fulton's condition factor: Setting the record straight. *Fisheries*, 31, 236–238.
- Nosil, P. (2012). *Ecological Speciation*. Oxford University Press.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). Vegan: Community ecology package. R Package Version 2.5–7. <https://CRAN.R-project.org/package=vegan>
- Perkins, T. A., Phillips, B. L., Baskett, M. L., & Hastings, A. (2013). Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. *Ecology Letters*, 16, 1079–1087.
- Phillips, B. L., Brown, G. P., & Shine, R. (2010). Evolutionarily accelerated invasions: The rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology*, 23, 2595–2601.
- Phillips, B. L., Brown, G. P., Travis, J. M. J., & Shine, R. (2008). Reid's paradox revisited: The evolution of dispersal kernels during range expansion. *The American Naturalist*, 172(Suppl 1), S34–S48.
- Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science*, 13, 288–294.
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Raeymaekers, J. A. M., Boisjoly, M., Delaire, L., Berner, D., Räsänen, K., & Hendry, A. P. (2010). Testing for mating isolation between ecotypes: Laboratory experiments with lake, stream and hybrid stickleback. *Journal of Evolutionary Biology*, 23, 2694–2708.
- Raeymaekers, J. A. M., Van Houdt, J. K. J., Larmuseau, M. H. D., Geldof, S., & Volckaert, F. A. M. (2007). Divergent selection as revealed by P(ST) and QTL-based F(ST) in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient. *Molecular Ecology*, 16, 891–905.
- Ravinet, M., Prodohl, P. A., & Harrod, C. (2013). On Irish stickleback: Morphological diversification in a secondary contact zone. *Evolutionary Ecology Research*, 15, 271–294.
- Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin/Fisheries Research Board of Canada*, 191, 1–382.
- Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology & Evolution*, 29, 233–242.
- Roy, D., Lucek, K., Walter, R. P., & Seehausen, O. (2015). Hybrid “super-swarm” leads to rapid divergence and establishment of populations during a biological invasion. *Molecular Ecology*, 24, 5394–5411.
- Rundle, H. D. (2002). A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution*, 56, 322–329.
- Schluter, D. (1995). Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. *Ecology*, 76, 82–90.
- Schluter, D. (2000). *The ecology of adaptive radiation*. OUP Oxford.
- Schmid, D. W., McGee, M. D., Best, R. J., Seehausen, O., & Matthews, B. (2019). Rapid divergence of predator functional traits affects prey composition in aquatic communities. *The American Naturalist*, 193, 331–345.
- Selz, O. M., & Seehausen, O. (2019). Interspecific hybridization can generate functional novelty in cichlid fish. *Proceedings of the Biological Sciences*, 286, 20191621.
- Shi, J., Macel, M., Tielbörger, K., & Verhoeven, K. J. F. (2018). Effects of admixture in native and invasive populations of *Lythrum salicaria*. *Biological Invasions*, 20, 2381–2393.
- Smyser, T. J., Tabak, M. A., Sloomaker, C., Robeson, M. S., 2nd, Miller, R. S., Bosse, M., et al. (2020). Mixed ancestry from wild and domestic lineages contributes to the rapid expansion of invasive feral swine. *Molecular Ecology*, 29, 1103–1119.
- Strait, J. T., Eby, L. A., Kovach, R. P., Muhlfeld, C. C., Boyer, M. C., Amish, S. J., Smith, S., Lowe, W. H., & Luikart, G. (2021). Hybridization alters growth and migratory life-history expression of native trout. *Evolutionary Applications*, 14, 821–833.
- Thompson, C. J., Ahmed, N. I., Veen, T., Peichel, C. L., Hendry, A. P., Bolnick, D. I., & Stuart, Y. E. (2017). Many-to-one form-to-function mapping weakens parallel morphological evolution. *Evolution*, 71, 2738–2749.
- Thompson, K. A., Peichel, C. L., Rennison, D. J., & McGee, M. D. (2021). Genetic evidence for environment-dependent hybrid incompatibilities in threespine stickleback. *bioRxiv*.
- Thompson, K. A., Peichel, C. L., Rennison, D. J., McGee, M. D., Albert, A. Y. K., Vines, T. H., Greenwood, A. K., Wark, A. R., Brandvain, Y., Schumer, M., & Schluter, D. (2022). Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. *PLoS Biology*, 20, e3001469.
- Thompson, K. A., & Schluter, D. (2022). Heterosis counteracts hybrid breakdown to forestall speciation by parallel natural selection. *Proceedings of the Biological Sciences*, 289, 20220422.
- Thompson, K. A., Urquhart-Cronish, M., Whitney, K. D., Rieseberg, L. H., & Schluter, D. (2021 Mar). Patterns, Predictors, and Consequences of Dominance in Hybrids. *The American Naturalist*, 197(3), E72–E88.
- Twining, C. W., Bernhardt, J. R., Derry, A. M., Hudson, C. M., Ishikawa, A., Kabeya, N., Kainz, M. J., Kitano, J., Kowarik, C., Ladd, S. N., Leal, M. C., Scharnweber, K., Shipley, J. R., & Matthews, B. (2021). The evolutionary ecology of fatty-acid variation: Implications for consumer adaptation and diversification. *Ecology Letters*, 4, 1709–1731.
- Vamosi, S. M., Hatfield, T., & Schluter, D. (2000). A test of ecological selection against young-of-the-year hybrids of sympatric sticklebacks. *Journal of Fish Biology*, 57, 109–121.
- van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H., & Hodgins, K. A. (2017). Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia. *Molecular Ecology*, 26, 5421–5434.
- Wagner, N. K., Ochocki, B. M., Crawford, K. M., Compagnoni, A., & Miller, T. E. X. (2017). Genetic mixture of multiple source populations accelerates invasive range expansion. *The Journal of Animal Ecology*, 86, 21–34.

- Wei, X., & Zhang, J. (2018). The optimal mating distance resulting from heterosis and genetic incompatibility. *Science Advances*, 4, eaau5518.
- Whitlock, M. C., Ingvarsson, P. K., & Hatfield, T. (2000). Local drift load and the heterosis of interconnected populations. *Heredity*, 84, 452–457.

#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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